Sevoflurane abolishes oxygenation impairment in a long-term rat model of acute lung injury

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Abstract: BACKGROUND Patients experiencing acute lung injury (ALI) often need mechanical ventilation for which sedation may be required. In such patients, usually the first choice an intravenously administered drug. However, growing evidence suggests that volatile anesthetics such as sevoflurane are a valuable alternative. In this study, we evaluate pulmonary and systemic effects of long-term (24-hour) sedation with sevoflurane compared with propofol in an in vivo animal model of ALI. METHODS Adult male Wistar rats were subjected to ALI by intratracheal lipopolysaccharide (LPS) application, mechanically ventilated and sedated for varying intervals up to 24 hours with either sevoflurane or propofol. Vital parameters were monitored, and arterial blood gases were analyzed. Inflammation was assessed by the analysis of bronchoalveolar lavage fluid (BALF), cytokines (monocyte chemoattractant protein-1 [MCP-1], cytokine-induced neutrophil chemoattractant protein-1 [CINC-1], interleukin [IL-6], IL-12/12a, transforming growth factor- , and IL-10) in blood and lung tissue and inflammatory cells. The alveolocapillary barrier was indirectly assessed by wet-to-dry ratio, albumin, and total protein content in BALF. Results are presented as mean ± standard deviation. RESULTS After 9 hours of ventilation and sedation, oxygenation index was higher in the LPS/sevoflurane (LPS-S) than in the LPS/propofol group (LPS-P) and reached 400 ± 67 versus 262 ± 57 mm Hg after 24 hours ($P < .001$). Cell count in BALF in sevoflurane-treated animals was lower after 18 hours ($P = .001$) and 24 hours ($P < .001$) than in propofol controls. Peak values of CINC-1 and IL-6 in BALF were lower in LPS-S versus LPS-P animals (CINC-1: 2.7 ± 0.7 vs 4.0 ± 0.9 ng/mL; IL-6: 9.2 ± 2.3 vs 18.9 ± 7.1 pg/mL, both $P < .001$), whereas IL-10 and MCP-1 did not differ. Also messenger RNAs of CINC-1, IL-6, IL-12a, and IL-10 were significantly higher in LPS-P compared with LPS-S. MCP-1 and transforming growth factor- showed no differences. Wet-to-dry ratio was lower in LPS-S (5.4 ± 0.2 vs 5.7 ± 0.2, $P = .016$). Total protein in BALF did not differ between P-LPS and S-LPS groups. CONCLUSIONS Long-term sedation with sevoflurane compared with propofol improves oxygenation and attenuates the inflammatory response in LPS-induced ALI. Our findings suggest that sevoflurane may improve lung function when used for sedation in patients with ALI.

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Sevoflurane Abolishes Oxygenation Impairment in a Long-Term Rat Model of Acute Lung Injury

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BACKGROUND: Patients experiencing acute lung injury (ALI) often need mechanical ventilation for which sedation may be required. In such patients, usually the first choice an intravenously administered drug. However, growing evidence suggests that volatile anesthetics such as sevoflurane are a valuable alternative. In this study, we evaluate pulmonary and systemic effects of long-term (24-hour) sedation with sevoflurane compared with propofol in an in vivo animal model of ALI.

METHODS: Adult male Wistar rats were subjected to ALI by intratracheal lipopolysaccharide (LPS) application, mechanically ventilated and sedated for varying intervals up to 24 hours with either sevoflurane or propofol. Vital parameters were monitored, and arterial blood gases were analyzed. Inflammation was assessed by the analysis of bronchoalveolar lavage fluid (BALF), cytokines (monocyte chemoattractant protein-1 [MCP-1], cytokine-induced neutrophil chemoattractant protein-1 [CINC-1], interleukin [IL-6], IL-12/12a, transforming growth factor-β, and IL-10) in blood and lung tissue and inflammatory cells. The alveolocapillary barrier was indirectly assessed by wet-to-dry ratio, albumin, and total protein content in BALF. Results are presented as mean ± standard deviation.

RESULTS: After 9 hours of ventilation and sedation, oxygenation index was higher in the LPS/sevoflurane (LPS-S) than in the LPS/propofol group (LPS-P) and reached 400 ± 67 versus 262 ± 57 mm Hg after 24 hours ($P < .001$). Cell count in BALF in sevoflurane-treated animals was lower after 18 hours ($P = .001$) and 24 hours ($P < .001$) than in propofol controls. Peak values of CINC-1 and IL-6 in BALF were lower in LPS-S versus LPS-P animals (CINC-1: 2.7 ± 0.7 vs 4.0 ± 0.9 ng/mL; IL-6: 9.2 ± 2.3 vs 18.9 ± 7.1 pg/mL, both $P < .001$), whereas IL-10 and MCP-1 did not differ. Also messenger RNAs of CINC-1, IL-6, IL-12a, and IL-10 were significantly higher in LPS-P compared with LPS-S. MCP-1 and transforming growth factor-β showed no differences. Wet-to-dry ratio was lower in LPS-S (5.4 ± 0.2 vs 5.7 ± 0.2, $P = .016$). Total protein in BALF did not differ between P-LPS and S-LPS groups.

CONCLUSIONS: Long-term sedation with sevoflurane compared with propofol improves oxygenation and attenuates the inflammatory response in LPS-induced ALI. Our findings suggest that sevoflurane may improve lung function when used for sedation in patients with ALI. (Anesth Analg 2016;XXX:00–00)

Acute lung injury (ALI) frequently requires both mechanical ventilation and sedation in the intensive care unit (ICU).[1,2] Currently, approximately 10% of patients treated in the ICU experience ALI or acute respiratory distress syndrome (ARDS),[3] and mortality rates of up to 50% have been described.[4] Immune activation and a massive release of proinflammatory mediators are hallmarks of ALI and ARDS,[5] and mechanical ventilation may further exacerbate the inflammatory response.[6–8] Cytokines recruit effector cells, which amplify the inflammatory response.[9]

Bacterial lipopolysaccharides (LPSs) are complex glycolipids found on the outer membrane of Gram-negative bacteria[10] and have been utilized to examine in vitro and in vivo inflammatory responses.[10–12] Other markers of ALI include endothelial barrier disruption, recruitment and transmigration of neutrophils, and development of a protein- and neutrophil-rich (noncardiogenic) pulmonary edema.[13–16]

According to current guidelines, intravenous propofol, midazolam, and dexmedetomidine are recommended for sustained sedation in critically ill patients.[17,18] However, since the introduction of the Anaesthetic Conserving Device (AnaConDa; Sedana Medical AB, Uppsala, Sweden), ICU sedation may also be possible with volatile anesthetics such as isoflurane or sevoflurane.[19] When compared with intravenous sedation, volatile sedation has been associated with shorter weaning times,[20] less ICU and hospital days,[21] and no difference in safety.[20,22,23]

The anti-inflammatory properties of volatile anesthetics have been demonstrated in experimental (LPS-induced) lung injury.[12] However, little is known about the impact of sedatives on the course of ALI during mechanical

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The authors declare no conflicts of interest.

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ventilation. We thus evaluated the effect of 2 sedative agents (sevoflurane or propofol) on pulmonary inflammation and lung function in ventilated and sedated rats with ALI for up to 24 hours. We hypothesized that sedation with sevoflurane would result in better oxygenation and less inflammation when compared with propofol.

METHODS

Animals

Adult male Wistar rats (Charles River, Sulzfeld, Germany; 364 ± 28 g) were used in this experiment (protocol approved by the veterinarian authorities, Veterinäramt Zürich, Switzerland, No. 94/2013). Animals were housed in standard cages at 22°C (±1°C), a 12-/12-hour light/dark cycle, and free access to food and water.

Animal Preparation

Anesthesia was induced with 100 mg/kg body weight (BW) thiopental (Pentothal; Inresa, Freiburg, Switzerland) intraperitoneally. Sterile polyethylene catheters were placed into the left jugular vein for fluid (Ringer's lactate, Ri-Lac; B. Braun, Sempach, Switzerland) and propofol administration (Disoprivan 2%; AstraZeneca, Zug, Switzerland) and into the right carotid artery for pressure monitoring and arterial blood gas analyses (aBGA). Oxygen saturation was recorded using pulse oximetry. After tracheotomy, rats were mechanically ventilated (Servo Ventilator 300; Maquet, Solna, Sweden) with or without sevoflurane (Sevorane; AbbVie AG, Baar, Switzerland). The ventilator was set to a peak inspiratory pressure of 14 cm H_2O, a positive end-expiratory pressure of 3 cm H_2O, an inspiratory fraction of oxygen (F_{IO_2}) starting at 0.6, and a respiratory rate of 30 breaths/min. Respiratory rate and F_{IO_2} were adjusted according to aBGAs (targets: P_{CO_2} 35–50 mm Hg, P_{O_2} >100 mm Hg, and pH 7.35–7.45; a maximum F_{IO_2} of 0.7 was used in our setting). Normal body temperature was maintained by the use of a heating pad with a feedback control system.

Assignment to Groups and Sedation Procedure

Groups of 2 animals were sedated for 6, 12, 18, or 24 hours before analysis (Figure 1) and randomly assigned to the following groups:

1. PBS-P (n = 16): pulmonary phosphate-buffered saline (PBS) instillation, propofol sedation
2. LPS-P (n = 24): LPS instillation, propofol sedation
3. PBS-S (n = 16): PBS instillation, sevoflurane sedation
4. LPS-S (n = 24): LPS instillation, sevoflurane sedation

Animals were sedated with 10 mg/kg BW/h propofol (PBS-P and LPS-P) or with 0.5 minimum alveolar concentration (approximately 1.3 vol%) sevoflurane (PBS-S and LPS-S groups). The dose of the 2 anesthetics was adjusted according to the blood pressure in animals (mean arterial pressure [MAP] target range: 60–80 mm Hg). For estimation of sedation depth, we defined rapid MAP peaks in combination with heart rate step-ups as a sign of insufficient or even plain level of anesthesia. In addition, whisker movement was seen when anesthesia was too low. In conclusion, continuous propofol or sevoflurane administration was increased.

Further information about the hemodynamic and analgesic management is provided in Supplemental Digital Content, Supplements S1 and S2 (http://links.lww.com/AA/B478; http://links.lww.com/AA/B479).

Induction of ALI

Twenty minutes after anesthesia induction, 0.3 µg/g BW LPS (*Escherichia coli*—LPS, serotype 055:B5; Sigma-Aldrich, Buchs, Switzerland) diluted in 300 µL PBS (Gibco, Paisley, UK) was applied intratracheally using a fine polyethylene tube (ID 0.58 mm; Smiths Medical International Ltd., Kent, UK). Control groups received 300 µL PBS only.

Collecting of Samples

aBGAs were performed before LPS or PBS instillation and every 3 hours subsequently until the end of the experiment. Whole blood was heparinized and allowed to sit for 5 minutes before being centrifuged (1250 relative centrifugal force [rcf], 4°C). For bronchoalveolar lavage, the right lung was 3 times flushed with 5 mL PBS. The collected fluid (bronchoalveolar lavage fluid [BALF]) was centrifuged (1250 rcf, 4°C). Cell pellets were resuspended in 1 mL PBS.

Snap frozen lungs, plasma, and BALF were stored at −80°C. In animals assigned for the determination of lung permeability, no bronchoalveolar lavage was performed.

Alveolocapillary Integrity

Oxygenation index (Horowitz index) was calculated using the quotient of arterial partial pressure (in mm Hg) and inspiratory fraction of oxygen in the ventilated lungs (P_{aO_2}/F_{IO_2}).

Total protein in BALF was measured using the dDC Protein Assay (Bio-Rad Laboratories, Hercules, CA) in accordance with the manufacturer's instructions. Albumin in BALF was determined by enzyme-linked immunosorbent assay (ELISA; Bethyl Laboratories Inc., Montgomery, TX) in accordance with the manufacturer's protocol.

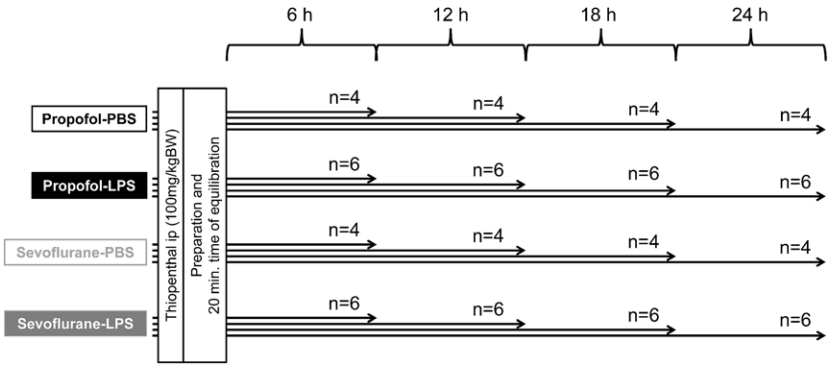

Figure 1. Experimental setup. After an initial i.p. sedation using thiopental for preparation of animals, acute lung injury was induced by intratracheal installation of lipopolysaccharide (LPS). Control animals, sedated with propofol or sevoflurane, were treated with phosphate-buffered saline, PBS) instead of LPS.

Lungs of animals sedated for 12 hours and stimulated with LPS were weighed immediately after removal and after 72 hours of drying at 65°C. The quotient of (lung wet weight)/(lung dry weight) was determined.

Inflammatory Cells in ALI

Resuspended cells of BALF were dyed with trypan blue and quantified with a Neubauer Chamber (C-Chip Neubauer Improved; Digital Bio, Pleasanton, CA). Cell differentiation is given in the Supplemental Digital Content, Supplement S3 (http://links.lww.com/AA/B480).

Inflammatory Cytokines

Sandwich ELISAs for monocyte chemoattractant protein-1 (MCP-1), cytokine-induced neutrophil chemoattractant protein-1 (CINC-1), interleukin-6 (IL-6), IL-12, and anti-inflammatory protein IL-10 were performed in accordance with the manufacturer's protocol to assess inflammatory responses in BALF and in plasma. Details are shown in the Supplemental Digital Content, Supplement S4 (http://links.lww.com/AA/B481).

Messenger RNA of Cytokines

Total RNA for the determination of MCP-1, CINC-1, IL-6, IL-12a, transforming growth factor-β, and IL-10 was isolated from lung tissue.

Detailed procedures, primers, and TaqMan probes are declared in the Table and the Supplemental Digital Content, Supplement S5 (http://links.lww.com/AA/B482).

Renal Function

Because of its metabolites, the long-term use of sevoflurane could result in renal disturbances and damages.[24] To address this question, we recorded urine output, total fluid administration, and net fluid balance of animals sedated for 24 hours in addition to the specific functional renal parameters creatinine in plasma and neutrophil gelatinase-associated lipocalin (NGAL) in urine. Details of the methods and kits can be found in Supplemental Digital Content, Supplement S6 (http://links.lww.com/AA/B483).

Statistical Analysis

Based on an effect size of $f^2 = 0.15$, an α value of .05, and a power of 80% in addition to 3 predictors: time, LPS/PBS and sevoflurane/propofol, and power analysis, revealed the inclusion of 80 animals.

Statistical analyses were performed in GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA) and Program R (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as mean ± standard deviation. For non-repeated measurements, 2-way analysis of variance was performed, and multiple comparisons were corrected for by the Tukey post hoc test.

Repeated measurements and comparison of experiments with different duration, linear mixed-effects models were used with PBS-P as the reference group. We applied linear mixed-effects models including the interaction term between time and group (no intercept) using the lme4 package. To test for time-specific group effects (testing for differences among groups at each time point), we subsequently applied a Dunnett post hoc test controlled for multiple testing using the generalized linear hypothesis test (glht) in the multcomp package. Specifically, linear mixed models were used for the analysis of hemodynamic stability measurements, continuous Ringer's lactate application, cumulative Ringer's lactate boluses, and for continuous norepinephrine application.

Wet-to-dry weight differences were assessed by Mann-Whitney *U* test (group size of 5 animals); the exact *P* value was computed (GraphPad Prism).

$P < .05$ was considered significant for Mann-Whitney *U* test and for linear mixed models. For Tukey-corrected analysis of variance, $P < .01$ was considered significant. The 99% Tukey-corrected confidence intervals (99% CIs) were used.

Table. Real-Time Quantitative TaqMan PCR—Used Primers and Probes

Gene	Primer Sequence	Length of Amplicon, nt
IL-12a		
Up	5′ CCT GCC AAG TGT CTT AAC CAG 3′	95
Down	5′ GTG CAA GAG TAA TGT TTC AAT TTC TC 3′	
Probe 62	5′ ACC TGC TG 3′	
IL-10		
Up	5′ CAG ATT CCT TAC TGC AGG ACT TTA 3′	128
Down	5′ CAA ATG CTC CTT GAT TTC TGG 3′	
Probe 13	5′ AGG CAG AG 3′	
IL-6		
Up	5′ CCC TTC AGG AAC AGC TAT GAA 3′	74
Down	5′ ACA ACA TCA GTC CCA AGA AGG 3′	
Probe 20	5′ CTG GCT GG 3′	
CINC-1		
Up	5′ CAC ACT CCA ACA GAG CAC CA 3′	120
Down	5′ TGA CAG CGC AGC TCA TTG 3′	
Probe 49	5′ CAG CCA CC 3′	
MCP-1		
Up	5′ AGC ATC CAC GTG CTG TCT C 3′	78
Down	5′ GAT CAT CTT GCC AGT GAA TGA GT 3′	
Probe 62	5′ ACC TGC TG 3′	
TGF-β		
Up	5′ AAG GGC TAC CAT GCC AAC TT 3′	92
Down	5′ TGG TTG TAG AGG GCA AGG AC 3′	
Probe 116	5′ GAG CCT GG 3′	
18S		
Up	5′ AAT CAG TTA TGG TTC CTT TGT CG 3′	65
Down	5′ GCT CTA GAA TTA CCA CAG TTA TCC AA 3′	
Probe 55	5′ TCC TCT CC 3′	

Abbreviations: 18S, housekeeping gene; CINC-1, cytokine-induced neutrophil chemoattractant-1; IL-6, interleukin 6; IL-10, interleukin 10; IL-12a, interleukin 12a; MCP-1, monocyte chemoattractant protein-1; nt, nucleotides; PCR, polymerase chain reaction; TGF-β, transforming growth factor beta.

RESULTS

Blood Oxygenation

Blood oxygenation decreased in both LPS groups over time, but the decrease was more pronounced in propofol animals. After 9 hours of injury, differences between LPS-P and LPS-S animals were observed (412 ± 70 vs 468 ± 34 mm Hg; 99% CI, −106 to −5 mm Hg; $P = .004$) that persisted at 24 hours (262 ± 57 vs 400 ± 67 mm Hg; 99% CI, −224 to −52 mm Hg; $P < .001$; Figure 2).

F_{IO_2} was adjusted in 5 animals in the LPS-P and in 2 animals in the LPS-S group. The maximal F_{IO_2} was 0.7 in the LPS-P and 0.65 in the LPS-S group.

Impairment of the Alveolocapillary Barrier

For the determination of lung wet-to-dry weight ratio, the time point of 12 hours was chosen because of the difference in oxygenation we observed. The wet-to-dry ratio of LPS-P was higher compared with LPS-S animals (5.7 ± 0.2 vs 5.4 ± 0.2; $P = .016$; Figure 3A).

To further explore the effect of anesthetic agent on the integrity of the alveolocapillary barrier, we measured total protein levels in BALF. When compared with propofol, sevoflurane had a nonsignificant effect on total protein in LPS-treated rats (1.5 vs 0.7 mg/mL; 99% CI, −0.1 to 1.7 mg/mL; $P = .028$, nonsignificant; Figure 3B). A similar pattern was observed for albumin concentrations in BALF (Figure 3C).

Cells in BALF increased over time in all LPS groups. After 18 hours, we found 44 ± 5 versus 28 ± 10 × 10^6 cells/mL BALF and after 24 hours 50 ± 14 versus 28 ± 7 × 10^6 cells/mL in LPS-P-treated versus LPS-S-treated animals, respectively (18 hours: 99% CI, 2.7 to 29.4 × 10^6 cells/mL; $P = .001$; 24 hours: 99% CI, 8.5 to 35.2 × 10^6 cells/mL; $P < .001$; Figure 3D).

Differential analysis of the cell types found in BALF revealed a higher count of neutrophils in LPS-P versus LPS-S (18 hours: 41 ± 4 vs 26 ± 11 × 10^6 cells/mL BALF; 99% CI, 1 to 29 × 10^6 cells/mL; $P = .006$; 24 hours: 47 ± 13 vs 25 ± 7 × 10^6 cells/mL BALF; 99% CI, 9 to 36 × 10^6 cells/mL; $P < .001$). No difference could be found for the subpopulations of macrophages, lymphocytes, or erythrocytes ($P = .41$, $P = .77$, and $P = .99$, respectively, at 24 hours).

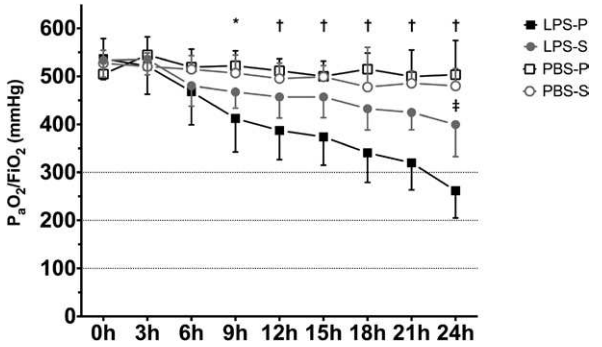

Figure 2. Oxygenation index (Horowitz). Oxygenation index in ventilated rats subjected to acute lung injury by intratracheal lipopolysaccharide (LPS) application. Control animals received phosphate-buffered saline (PBS). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. Values are expressed as mean ± SD. *$P = .004$, †$P \leq .001$ LPS-P versus LPS-S at the corresponding time point; ‡$P = .004$ LPS-S versus PBS-S at 24 hours. Fio_2, inspiratory fraction of oxygen.

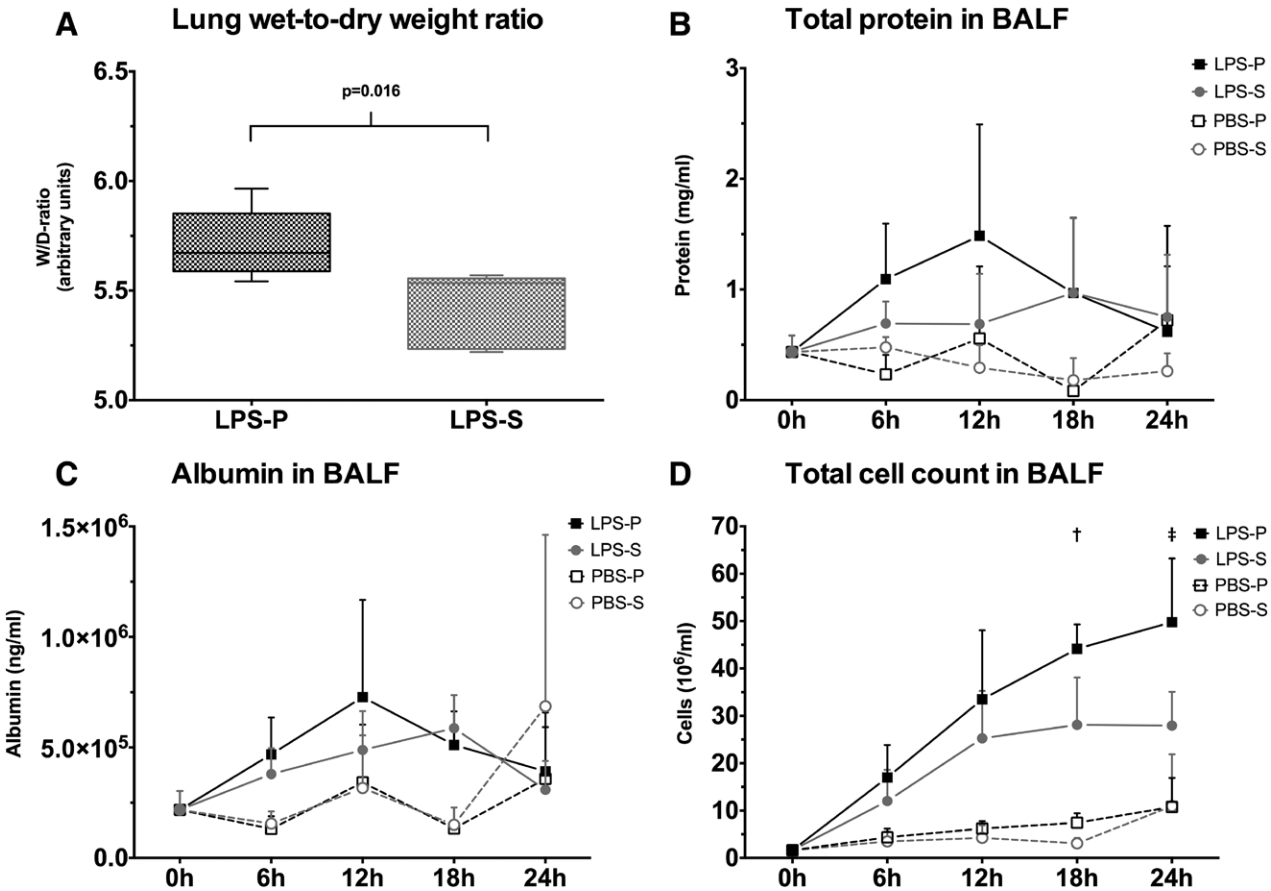

Figure 3. A–D, Determination of integrity of the alveolocapillary barrier. The alveolocapillary barrier was evaluated by the determination of total protein content (B) and albumin (C) in bronchoalveolar lavage fluid (BALF) obtained from ventilated rats subjected to acute lung injury (ALI) by intratracheal lipopolysaccharide (LPS) application (control animals received phosphate-buffered saline [PBS]). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. In addition, total cell count in BALF (D) and lung wet-to-dry-ratio (W/D ratio, A) were assessed. Values are expressed as mean ± SD. †$P = .001$, ‡$P < .001$ LPS-P versus LPS-S at the corresponding time point.

Inflammation in the Respiratory Compartment

Twelve hours after LPS administration, MCP-1 concentrations were higher in both LPS-P and LPS-S groups compared with their respective controls. At 12 hours, a significant injury-induced increase of MCP-1 was observed (PBS-P versus LPS-P: 10 ± 11 versus 212 ± 148 ng/mL, 99% CI, −374 to −30, $P < .001$, and PBS-S versus LPS-S: 6 ± 2 versus 216 ± 182 ng/mL, 99% CI, −382 to −37, $P = .001$). Values did not differ between rats sedated with propofol or sevoflurane ($P = 1.0$). At 18 hours, MCP-1 concentrations were again not different between LPS-P versus LPS-S animals (246 ± 150 vs 218 ± 73 ng/mL, respectively; 99% CI, −125 to 183 ng/mL; $P = .9$; Figure 4A).

At 6 hours, CINC-1 values measured in BALF were higher in LPS-P than in LPS-S animals (4.0 ± 0.9 vs 2.7 ± 0.7 ng/mL, respectively; 99% CI, 0.4 to 2.3 ng/mL, $P < .001$; Figure 4B).

IL-6 concentrations in BALF at 6 hours were also higher in LPS-P than in LPS-S animals (18.9 ± 7.1 vs 9.2±2.3 ng/mL; 99% CI, 5.3 to 14.0 ng/mL; $P < .001$; Figure 4C). No differences were found at later time points ($P = .31$, .99, and .71 at 12, 18, and 24 hours).

IL-12 was below detection range in all groups at all time points. IL-10 concentrations in BALF did not differ at any time point between LPS-S and LPS-P groups ($P = .56$, .27, .35, and .90 at 6, 12, 18, and 24 hours, respectively; Figure 4D).

Inflammation in the Interstitial Compartment

The messenger RNA (mRNA) expression pattern for the proinflammatory cytokines MCP-1, CINC-1, IL-6, and IL-12a in lung tissue was similar (Figure 5, A–D). Although no difference between LPS-P and LPS-S was found at 6 hours for MCP-1 mRNA, CINC-1 mRNA values were higher in the LPS-P than in the LPS-S group (91 ± 33 vs 66 ± 16; 99% CI, 6 to 45; $P < .001$). Similarly, IL-6 mRNA expression was higher in LPS-P than in LPS-S animals at 6 hours (1483 ± 338 vs 923 ± 353; 99% CI, 309 to 812; $P < .001$) as were IL-12a mRNA values (4.3 ± 1.4 vs 2.6 ± 0.4; 99% CI, 0.9 to 2.7; $P < .001$). For transforming growth factor-β, the 2 sedative groups did not differ (Figure 5E).

IL-10 mRNA was clearly upregulated in the LPS-P group compared with LPS-S animals (6 hours: 41 ± 15 vs 23 ± 10; 99% CI, 3 to 33; $P = 0.001$; Figure 5F).

Systemic Inflammation

With our sample size, we did not detect a significant difference between LPS-P and LPS-S in plasma MCP-1 levels (6 hours: 429.9 ± 139.6 vs 343.6 ± 112.7 ng/mL; 99% CI, −107.3 to 278.0 ng/mL; $P = .48$; 18 hours: 384.7 ± 186.9 vs 220.0 ± 52.4 ng/mL; 99% CI, −29.0 to 358.3 ng/mL; $P = .04$; 24 hours: 448.9 ± 213.8 vs 277.5 ± 115.9 ng/mL; 99% CI, −22.2 to 365.1 ng/mL; $P = .03$; Figure 6A).

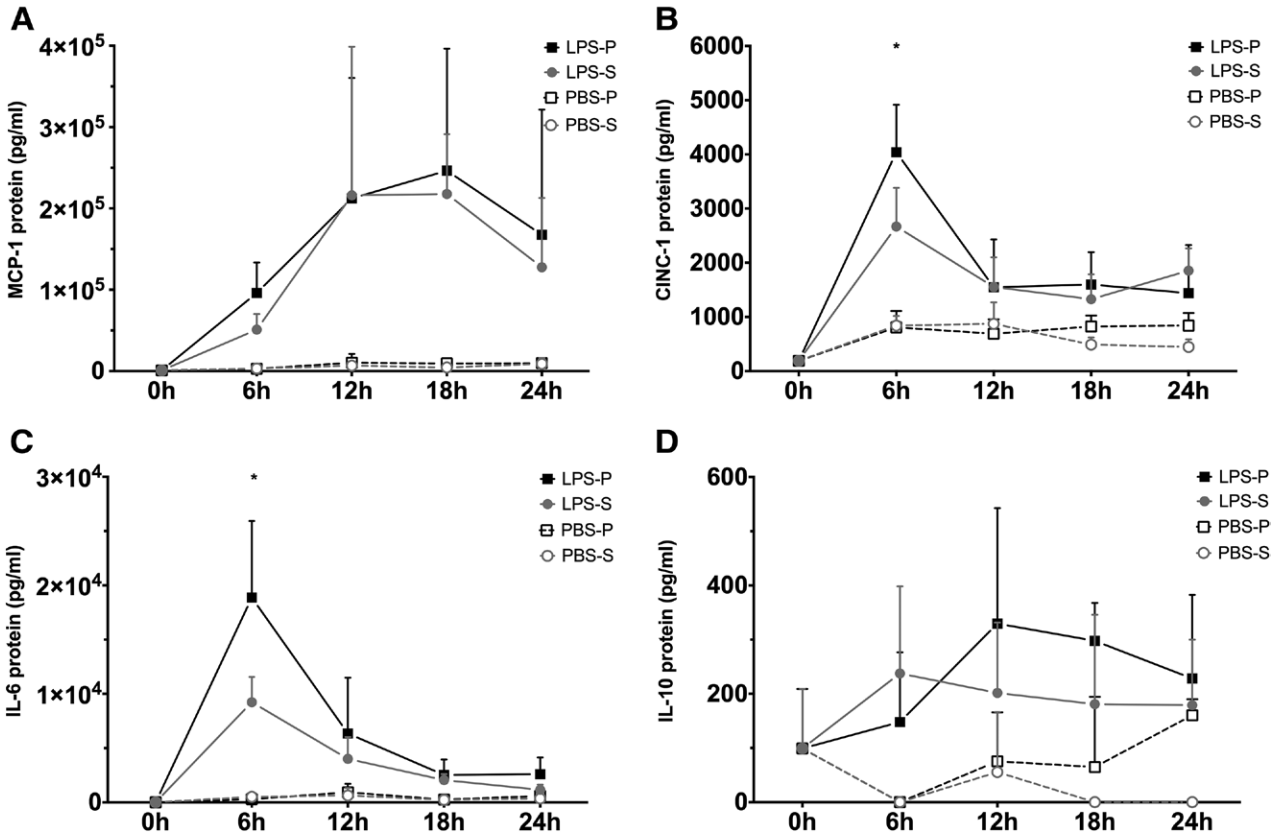

Figure 4. A–D, Pro- and anti-inflammatory mediators in bronchoalveolar lavage fluid (BALF). Assessment of the inflammatory response in the alveolar compartment by determination of proinflammatory cytokine such as monocyte chemoattractant protein-1 (MCP-1; A), cytokine-induced neutrophil chemoattractant-1 (CINC-1; B), interleukin-6 (IL-6; C), and the anti-inflammatory cytokine interleukin-10 (IL-10; D) in BALF from ventilated rats subjected to acute lung injury by intratracheal lipopolysaccharide (LPS) application (control animals received phosphate-buffered saline [PBS]). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. Values are expressed as mean ± SD. *$P < .001$ LPS-P versus LPS-S at the corresponding time point.

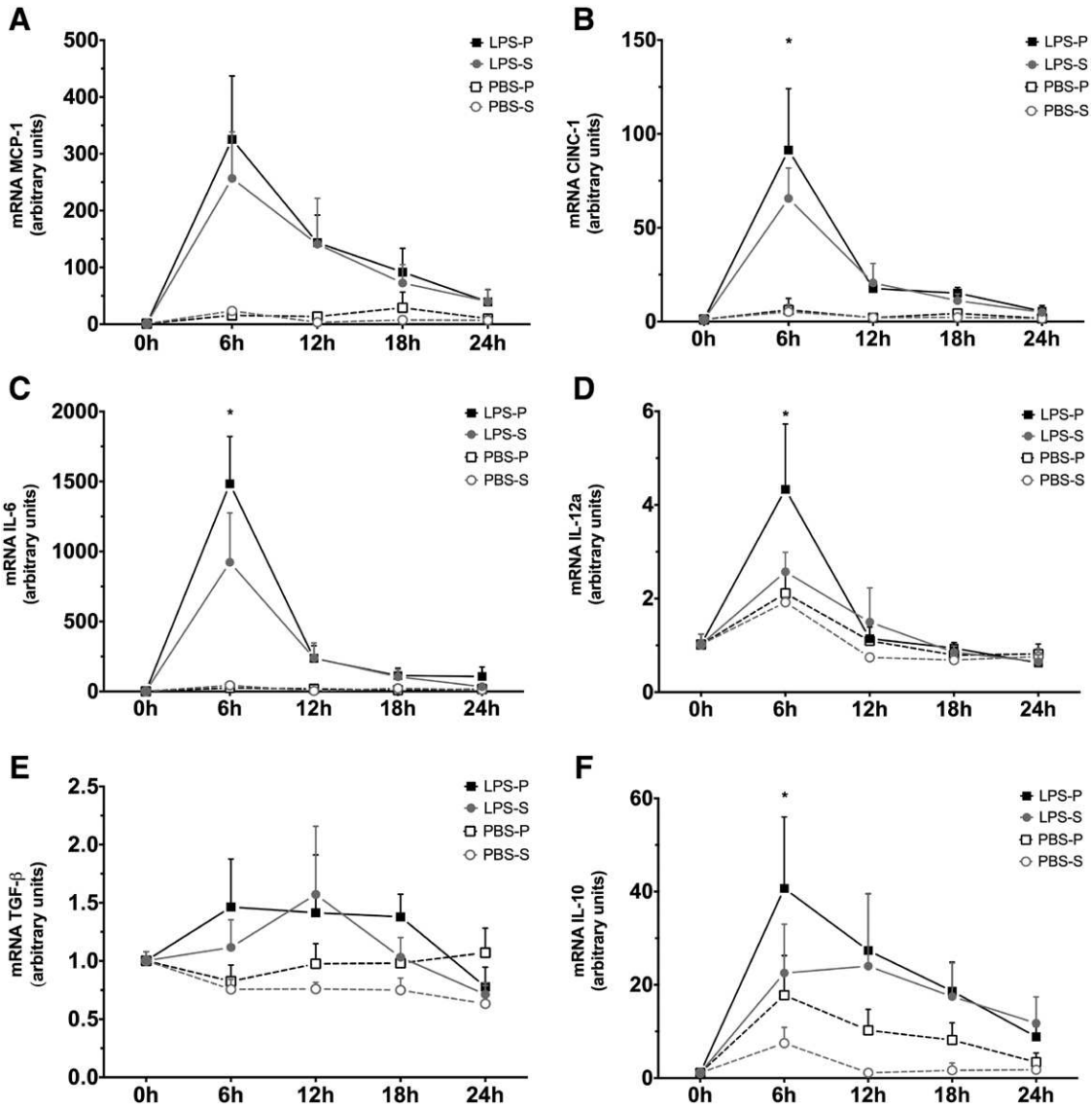

Figure 5. A–F, Pro- and anti-inflammatory mediators in lung tissue. Assessment of inflammation in the interstitial compartment by determination of messenger RNA of cytokines monocyte chemoattractant protein-1 (MCP-1; A), cytokine-induced neutrophil chemoattractant-1 (CINC-1; B), interleukin-6 (IL-6; C) and interleukin-12a (IL-12a; D), transforming growth factor-beta (TGF-β; E), and interleukin-10 (IL-10; F) in lung tissue from ventilated rats subjected to acute lung injury by intratracheal lipopolysaccharide (LPS) application (control animals received phosphate-buffered saline [PBS]). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. Values are expressed as mean ± SD. *P < .001 LPS-P versus LPS-S at the corresponding time point.

Plasma levels of CINC-1 at 6 hours after LPS challenge were also not different: LPS-P versus LPS-S: 6.7 ± 3.6 versus 4.8 ± 2.4 ng/mL; 99% CI, −0.4 to 4.2 ng/mL; P = .04 (Figure 6B). IL-6 and IL-10 levels in plasma were below the detection range of the ELISA kit.

Hemodynamic Stability

Blood pressure was similar in all animal groups over time (Figure 7).

Continuous Ringer's lactate application was evaluated in 30-minute intervals and was higher in the LPS-P versus the LPS-S group between 7.5 and 12.5 hours. The average fluid administered by continuous infusion within this period was 1.3 ± 0.1 versus 1.5 ± 0.2 mL/h. The following Dunnett-corrected P values for the 30-minute intervals were calculated: .037 (7.5–8 hours), .023 (8–8.5 hours), .024 (8.5–9 hours), .036 (9–9.5 hours), .056 (9.5–10 hours), .037 (10–10.5 hours), .037 (10.5–11 hours), .037 (11–11.5 hours), .023 (11.5–12 hours), and .036 (12–12.5 hours). Also, between 6 and 12 hours, cumulative application of Ringer's lactate boluses was higher in the LPS-P compared with the LPS-S group (1.7 ± 0.7 vs 2.6 ± 1.4 mL; Dunnett-corrected P = .004).

Continuous norepinephrine infusions were not different between groups except for the first hour of treatment where the norepinephrine dosage was lower in LPS-P-treated versus LPS-S-treated animals (0–30 minutes: 0.01 ± 0.03 vs 0.09 ± 0.05; P = .006; 30–60 minutes: 0.05 ± 0.07 vs 0.14 ± 0.07 μg/kg/min; Dunnett-corrected P < .001).

Renal Function

Overall fluid administration in animals treated for 24 hours was similar in all groups (PBS-P: 47.5 ± 4.8 mL; LPS-P: 51.2

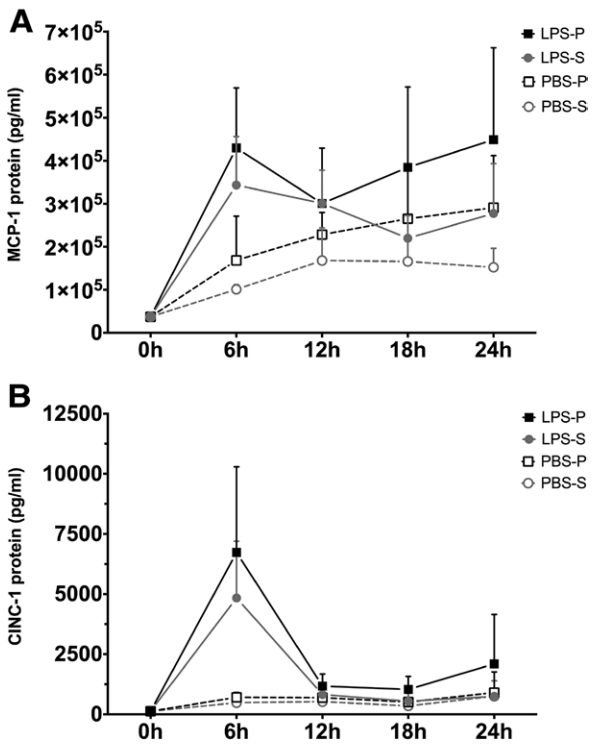

Figure 6. A and B, Proinflammatory mediators in plasma. Determination of systemic inflammation by measurement of the pro-inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1; A) and cytokine-induced neutrophil chemoattractant-1 (CINC-1; B) in plasma obtained from ventilated rats subjected to acute lung injury by intratracheal lipopolysaccharide (LPS) application; control animals received phosphate-buffered saline (PBS). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. Values are expressed as mean ± SD.

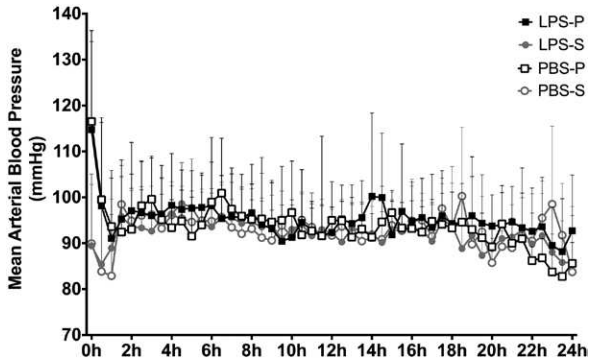

Figure 7. Mean arterial blood pressure determined by a catheter in the right carotid artery in ventilated rats subjected to acute lung injury by intratracheal lipopolysaccharide (LPS) application (control animals received phosphate-buffered saline [PBS]). Animals were sedated with sevoflurane (-S) or propofol (-P) for up to 24 hours. Values are expressed as mean ± SD.

± 4.2 mL; PBS-S: 51.0 ± 3.9 mL; LPS-S: 47.7 ± 4.2 mL; $P = .55$ for LPS-P vs LPS-S). Overall fluid balance (fluid administration minus urine output) was not different between groups (Figure 8A).

Creatinine values in animals sedated for 24 hours in the LPS-P group did not differ from those in the LPS-S group (70 ± 59 vs 24 ± 11 μmol/L; 99% CI, −11 to 103 μmol/L; $P = .05$)

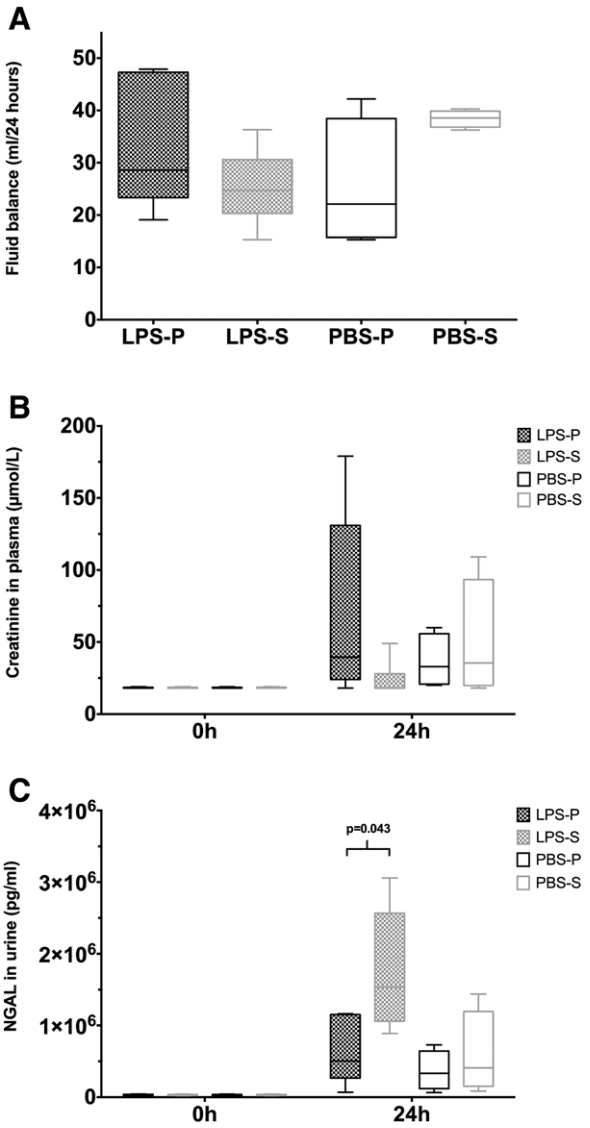

Figure 8. A–C, Renal parameters. Fluid balance after 24 hours (A) and renal parameters creatinine in blood (B) as well as neutrophil gelatinase-associated lipocalin, neutrophil gelatinase-associated lipocalin (NGAL; C) in urine derived from ventilated rats subjected to acute lung injury (ALI) by intratracheal lipopolysaccharide (LPS) application (control animals received phosphate-buffered saline [PBS]). Animals were sedated with propofol (-P) or sevoflurane (-S) for up to 24 hours. Data are expressed as box plots with mean ± maximum and minimum.

or from either control groups (PBS-P: 37 ± 16 μmol/L; 99% CI, −97 to 31 μmol/L; $P = .32$, and PBS-S: 50 ± 36 μmol/L; 99% CI, −43 to 84 μmol/L; $P = .72$; Figure 8B).

After 24 hours, urine NGAL was not different between groups (668 ± 473 μg/mL in LPS-P animals compared with 1756 ± 821 μg/mL in LPS-S animals; 99% CI, −2456 to 278 μg/mL; $P = .043$; Figure 8C).

DISCUSSION

In this study, we found that rats with LPS-induced ALI had less biomarker evidence of inflammation, less histologic damage to their lung, and less functional impairment of lung function when sedated with sevoflurane than with propofol.

Sedation and mechanical ventilation in the ICU is often required in patients experiencing ALI.[25] Since the launch of the AnaConDa system, the use of sevoflurane and isoflurane has become a more feasible option in Europe, but is still an off-label use in many countries, including the United States.[26,27]

The effect of sedation type on the course of ALI is unknown. In this study, we found improved oxygenation and an attenuated pulmonary inflammatory response when sevoflurane was used for sedation versus propofol.

When compared with propofol sedation, sevoflurane attenuated the production of proinflammatory cytokines IL-6, CINC-1, and reduced inflammatory cell counts in BALF. In lung tissue, representing the interstitial compartment, mRNA of IL-6, CINC-1, and IL-12a was lower with sevoflurane than with propofol sedation. Similarly, in the vascular compartment, assessed by inflammatory mediators in the plasma, CINC-1 and MCP-1 were lower in animals sedated with sevoflurane, even not significant.

Our results are in accordance with several previous animal and clinical studies focusing on short-term volatile sedation or anesthesia. In a 2009 study, Voigtsberger et al[12] observed improved oxygenation and attenuated inflammatory response in an LPS-induced ALI rat model after 6 hours when sevoflurane was used rather than propofol. Ferrando et al[28] endorsed the results of Voigtsberger et al[12] in a porcine 2-hit ARDS model. In addition, the anti-inflammatory effects of volatile anesthetics in the deflated–reventilated lung undergoing hypoxia–reoxygenation could also be verified in clinical investigations: De Conno et al[29] found that sevoflurane reduced pulmonary inflammatory response and chemoattraction of neutrophils in patients undergoing 1-lung ventilation for lung resection. Schilling et al[30] described similar results for a sevoflurane or desflurane anesthesia during 1-lung ventilation in the ventilated lung. In our study, we extended the duration of sedation to 24 hours, performed a more detailed investigation of the inflammation (in terms of time course and compartments), and supported blood pressure with norepinephrine to more closely simulate the ICU environment. Despite these changes, our results agree with those described earlier.

Of note is the only transient increased expression of pulmonary cytokines. One possible explanation is that in the clinical environment, Gram-negative bacteria play a crucial role, whereas in our model, we used LPS instead of live bacteria. The advantage of our model is a greater degree of reproducibility, but a potential disadvantage may be a rapid removal of LPS leading to only a temporary inflammatory effect. The cytokine release patterns found in our experiments, however, have been previously described in humans.[31] In an animal model of cecal ligation and puncture with vital bacteria as pathogens, a temporary upregulation of cytokines was observed as well.[32]

Another potential confounder in our study was the use of norepinephrine, which itself has immunomodulatory properties. Although norepinephrine doses were lower in the propofol group, the known pro- and anti-inflammatory effects of norepinephrine[33] may have affected our results. However, the difference was only during the first hour, making a meaningful effect on the rest of the study unlikely.

The difference in norepinephrine application might be explained by different sedation techniques where we observed a slower onset of sedation in the propofol group because of different administration routes. Other investigators performing ICU studies have also reported that initial use of the AnaConDa may cause transient hypotension.[34,35]

We observed a distinct improvement in oxygenation in animals sedated with sevoflurane. This interesting and clinically relevant finding may be a result of an attenuated inflammation (as proven by the current study) and thus less pulmonary edema. The lower wet-to-dry ratio we found in the sevoflurane group, together with the results of a previous in vivo ALI study in which sevoflurane affected edema formation rather than clearance,[36] supports this possibility. Beside an attenuation of inflammation, other factors may also contribute to an impaired oxygenation such as the development of atelectasis, which has been described in ventilated rats.[37]

For proper interpretation of the wet-to-dry ratio, hemodynamic and fluid management have to be taken into consideration. Fluid management was based on MAP, pulse pressure, urine output, and hematocrit. The LPS-P group received more fluid between 7.5 and 12 hours of sedation demonstrating an intergroup difference of 0.2 mL; although statistically significant, we consider this difference clinically not relevant. In addition, after 24 hours, overall fluid balance was similar between the 2 LPS groups.

The mechanism by which sevoflurane may affect pulmonary edema or inflammation is unclear. However, 1 possible link is the pulmonary γ-aminobutyric acid type A ($GABA_A$)-receptor (GABAAR).[38] Current studies suggest that modifying or blocking GABAAR might lead to an improved alveolar fluid clearance in ALI[39] and that GABAAR function may be modulated by isoflurane.[39] Further work is needed to better understand the role of GABAAR in volatile-mediated immunomodulation and alveolar fluid clearance. Another potential mediator for the sevoflurane effect we observed is downregulation of toll-like receptor 2 and toll-like receptor 4 resulting in an attenuation of proinflammatory cytokines.[40] Although not assessed in this current study, phosphorylation of extracellular-regulated kinase in macrophages on LPS stimulation may be another sevoflurane-mediated immunomodulatory mechanism.[41]

In contrast to previous studies, we found that pulmonary albumin content,[12,42] another integrity marker of the alveolocapillary barrier, was not different between propofol and sevoflurane groups. This observation may be because of lower LPS doses used in our study compared with previous studies: our dose was adapted to provoke a moderate inflammatory response allowing for a 24-hour observation period without deaths because of sustained hemodynamic instability. The type and formulation of propofol may also have played a role: Voigtsberger et al[12] used Cremophor as the pharmacologic carrier of propofol, whereas we used 1% propofol without ethylenediaminetetraacetic acid.

Volatile anesthetics exert a dose-dependent immunomodulation.[40,41] In our study, however, we targeted a sevoflurane dose that mimics long-term sedation in the ICU. For that, 0.5 minimal alveolar concentration sevoflurane is sufficient.[34,43] This dose may be too low, however, to demonstrate

pronounced effects on neutrophil and alveolocapillary barrier function as observed in previous studies.[41,42]

To our knowledge, our investigation is the first to demonstrate a significant reduction of the proinflammatory cytokine IL-12a by sevoflurane. Dendritic cells, neutrophils, and mononuclear cells are the major source of IL-12. IL-12 plays a pivotal role in the activation of natural killer cells and T-cells and the production of interferon-γ and other cytokines.[44] Elimination of IL-12 may reduce mortality and attenuate bacterial count in sepsis.[44] Further investigations will be needed to confirm whether or not an IL-12 reduction also leads to a survival benefit in ALI.

Because long-term sedation with sevoflurane may result in renal toxicity,[20,22] we monitored renal function in our study using NGAL and creatinine. Although we found higher NGAL levels in sevoflurane-treated animals, creatinine levels were not different between groups. More work is needed to assess the potential nephrotoxicity of long-term sevoflurane sedation.

Our study has several limitations: (1) results from an animal model may not simply be translated/transferred into a clinical setting; (2) an observation period of 24 hours with volatile sedation may still be too short because patients experiencing ALI usually require a much longer duration of mechanical ventilation and sedation; (3) LPS leads to a sterile infection, which may not reflect all aspects of a bacterial pulmonary infection; and (4) we adjusted ventilator settings during the experiment to achieve normoventilation, that is, normocapnia. In ALI and ARDS, permissive hypercapnia[45,46] is preferred over strict normoventilation, and hypercapnic acidosis may also affect inflammation and oxygenation.[47,48]

Avoidance of 1.0 F_{IO_2}, as performed in previous studies,[12] may also have influenced the results because of the time-dependent impact of oxygen on pulmonary damage and mortality.[49,50] We used a maximum F_{IO_2} of 0.7, however, which is consistent with clinical care.

In summary, our study demonstrates that prolonged sedation with sevoflurane in the context of ALI attenuates the pulmonary inflammatory response, decreasing attraction of inflammatory cells into alveoli and decreasing production of proinflammatory cytokines. Our findings suggest that the attenaute pulmonary inflammation also results in less pulmonary edema and improved oxygenation compared with propofol sedation. The current study is in accordance with previous short-term studies evaluating volatile sedation in the context of ALI. Further studies are needed to uncover possible mechanistic pathways involved in pulmonary protection and to address the question of transferability of our findings into a clinical setting.

DISCLOSURES

Name: Patrick Kellner, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Patrick Kellner has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Mattia Müller, MD.
Contribution: This author helped conduct the study, analyze the data, and write the manuscript.
Attestation: Mattia Müller has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Tobias Piegeler, MD.
Contribution: This author helped analyze the data and write the manuscript.
Attestation: Tobias Piegeler has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Philipp Eugster.
Contribution: This author helped conduct the study and analyze the data.
Attestation: Philipp Eugster has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Christa Booy.
Contribution: This author helped conduct the study, analyze the data, and write the manuscript.
Attestation: Christa Booy has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Martin Schläpfer, MD, MSc.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Martin Schläpfer has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Beatrice Beck-Schimmer, MD.
Contribution: This author helped design the study, analyze the data, and write the manuscript.
Attestation: Beatrice Beck-Schimmer has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

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